Serum Vitamin D_3 Level in a Sample of Healthy Students in Baghdad College of Medicine

Khudair Z Al-Bedri

ABSTRACT:

BACKGROUND:

Vitamin D has an important biologic activity including cellular proliferation, differentiation, immune system modulation and muscle strengthening. Vitamin D is considered as essential environmental factor in the etiology of T-cell mediated auto immune disease.

OBJECTIVE:

To estimate the level of vitamin D_3 in a sample of healthy students in Baghdad College of Medicine and correlation of the various variables to the levels of vitamin D_3.

SUBJECTS AND METHODS:

A cross–sectional study was conducted on 90 healthy Iraqi Students in Baghdad College of Medicine, from 1st March 2014 to 30th October 2014. Subjects had history of chronic illness, smoking, alcohol consumption, pregnancy, post-menopause and chronic use of medication was excluded from the study. Subjects were evaluated for age, gender, body mass index, dietary habit, walking exercise and graduate level of students (undergraduate or postgraduate). Venous blood samples were obtained from all subjects to estimate serum level of 25 hydroxy vitamin D_3 using enzyme –linked immunosorbent assay (ELISA).

RESULTS:

The frequency of female was more than males (63.3% versus 36.7%) with mean age of 32.24±9.59 years. Mean serum vitamin D_3 level was 18.33± 8.8 ng/ml. There was significant higher level of vitamin D_3 in the undergraduate student than postgraduate (22.29 ng/ml versus 15.93 ng/ml) with p value = 0.001. Subjects who consume vegetables rich diet had higher serum of vitamin D_3 than those who consume more meat and balanced diet (27.467, 16.975 ng/ml and 15.971 ng/ml) with p value <0.001. Subjects who used to exercise and perform daily exercise > 30 minutes per day have higher level of vitamin D_3 than subjects who exercised ≤ 30 minutes, and subject without exercise (29.27 ng/ml, 22.91 ng/ml and 16.34 ng/ml respectively) with p value < 0.00. Obese students have vitamin D3 level higher than over weight and normal weight students (25.85, 20.12 and 16.17 ng/ml respectively) with p value < 0.00. Gender did not exhibit statistical difference (male 18.42 ng/ml and female 18.28 ng/ml).

CONCLUSION:

Most studied subjects had mild to moderate vitamin D inadequacy. Subjects who consume more vegetable, perform brisk daily walking exercise, undergraduate and overweight have higher mean level of vitamin D_3 while the gender have no effect on mean level of vitamin D_3.

KEYWORDS: vitamin D_3, Iraqi students, diet, walking exercise.

INTRODUCTION:

Vitamin D comprises a group of fat soluble seco-sterols which is an essential element for establishing and maintaining bone structure, vitamin D level is best measured by the serum concentration of 25-hydroxyvitamin D level, the Institute of Medicine has recently recommended that serum 25(OH) D is adequate when it is higher than 20ng/ml, clinical vitamin D deficiency only occurs when serum 25 (OH) D is lower than10 ng/ml.[1]

Vitamin D status in an individual is dependent on numerous factors include genetic, life style and geographic factors, which is determined by sunshine exposure, a small part comes from dietary intake of oily fish and herring and oils from fish including cod liver oil. [2]

Turkey studies showed a strong relationship between Vitamin D status and clothing, with level of 22.4, 12.8, and 3.6ng/ml in women with western clothing, women with hijab and women with niqab respectively.[3]

Baghdad University, College of Medicine, Medical Department, Baghdad, Iraq.

Studies from Saudi Arabia showed the mean level of serum 25(OH) D was 26.4 ng/ml in women aged between 20-45years.[4]
The studies from Europe showed higher level of 25(OH) D in Norway and Sweden which may be due to a high intake of fatty fish and cod liver oil, but lower level in Spain, Italy and Greece which may be attributed to more skin pigmentation and sunshine avoidance.[5]

MATERIALS AND METHODS:

Study design:

This is a cross–sectional study conducted at Rheumatology Unit, Baghdad Teaching Hospital, and Baghdad, Iraq. It was carried out from 1st March 2014 till 30th October 2014. Serum concentration of vitamin D3 was measured in all participants. Informed consent was obtained from all participants and this study was approved by the ethical committee of Baghdad University, College of Medicine, and Department of Medicine.

Sample selection

A total of 90 students from Baghdad College of Medicine were included in the study. Individuals were excluding from the study if they had comorbid disease, chronic illness, pregnancy, chronic use of medication, history of smoking or alcohol consumption and post-menopausal women.

Data collection and laboratory measurements

We used paper clinical research from through interview and questionnaires. We asked the students about: age, sex, BMI [using the equation weight (kg) divided by height (m^2)], education level, diet habit and brisk walking exercise. Full history was taken and complete clinical examination of students was done. Weight and height of all students were recorded and body mass index (BMI) was calculated. Serum levels of 25 hydroxy vitamin D_3 were determined from each students using enzyme – linked Immunosorbent assay (ELISA). Cobas e 411 device was used from HITACHI (Roche Diagnostic GmbH), the kit was derived from Elecsys and Cobas e analyzers (Roche) with REF 05894913190 serial number. Serum vitamin D3 level < 10 ng/ml indicate severe deficiency, 10-25 ng/ml indicate mild to moderate deficiency and 26-80 ng/ml indicate optimum level.

Statistical methods:

Minitab® 16.1.1statiticall program for windows was used to compare the significance in the mean values in the comparison groups. Data were presented as mean ± SD (standard deviation). P value used considered significant if less than 0.05. ANOVA test were used for comparing different mean and post hoc Tukey test also used to assess between group variations. Student t test used to compare mean of two samples. Multiple linear regressions using dummy variables were used to assess the possibility of interaction of different variables.

RESULTS:

Baseline characteristics of individuals involved in the study is shown in Table (1). The majority participants were female (63.3%) with normal BMI (56.7%).

Table 1: Baseline characteristics of individuals involved in the study.

Parameter	Variables	Value		P value
		Number	Percentage	
Age (Years)	Mean ± SD	32.24 ± 9.59		
	Median	31.5		
Gender	Female	57	63.3	0.011
	Male	33	36.7	
BMI (Kg/m^2)	Normal	51	56.7	0.000
	Overweight	32	35.5	
	Obese	7	7.8	
	Mean ± SD	24.21 ± 3.58		
	Median	23.5		
Diet habit	Balanced	35	38.9	0.003
	Meat	40	44.4	
	Plant	15	16.7	
Walking Exercise	>30 min	8	8.9	0.000
	≤30 min	11	12.2	
	No exercise	71	78.9	
Level of education	Post graduate	56	62.2	0.020
	Under graduate	34	37.8	

SD= Standard deviation, P value significant < 0.05
MBI: body mass index

The level of vitamin D3 was located in the category of mild to moderate deficiency in majority of studied group (61.1%) as shown in table (2).

Table 2: Serum vitamin D3 levels.

Variable	Number	Percentage	P value
Optimal [6]	18	20	0.000
Mild to moderate [6]	55	61.1	
Severe [6]	17	18.9	
Mean ± SD	18.33 ± 8.8		
Median	17.0		

Relationship between serum vitamin D_3, BMI and Age

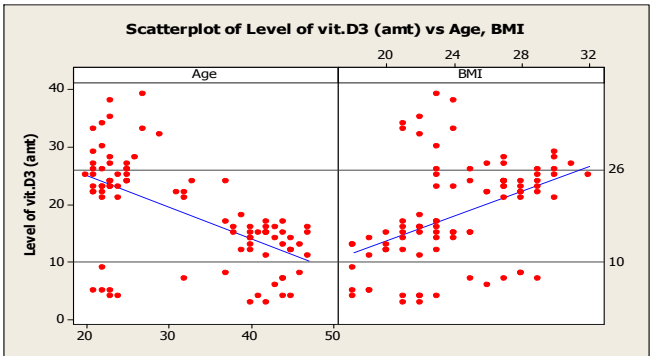

Figure 1: Correlation of vitamin D_3 with age and BMI.

Figure (1) shows correlation between age, BMI and serum vitamin D3 and it was highly significant with p value 0.000, in which there was a negative relationship between age and vitamin D3 that mean age increase by 1 year vitamin D3decrease by factor 0.463 and in the same time this was positive relationship with increasing by 1 unite of BMI there is increase in vitamin D3 level by factor 0.541.

Effect of walking exercise on serum vitamin D3 and its interaction with age and BMI

There was a correlation between level of exercise (when defined as ≤30min, > 30min, and without walking exercise), BMI, age to the level of serum vitamin D3.

Figure (2) shows that students on exercise > 30 min had the highest level of vitamin D_3, followed by those on exercise ≤30 min, but those without exercise had low level of serum vitamin D3.

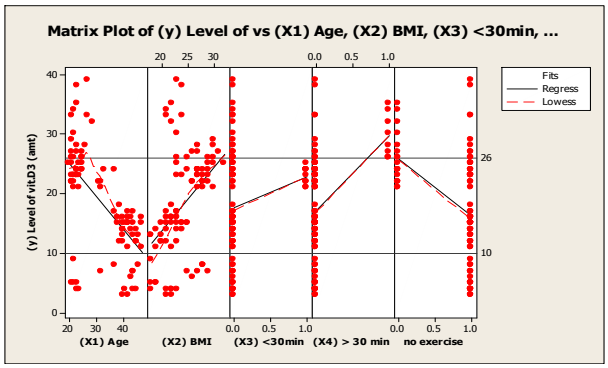

Figure 2: Correlation of vitamin D_3 with age, BMI, and level of exercise.

Although the students on brisk daily walking exercise had high level of vitamin D_3 but statistically was not significant.

Effect of gender on serum vitamin D3 and its interaction with age and BMI

There was a correlation between gender, age BMI and serum vitamin D3.

Figure (3) shows that the student's gender had similar effect on variation of serum vitamin D3.

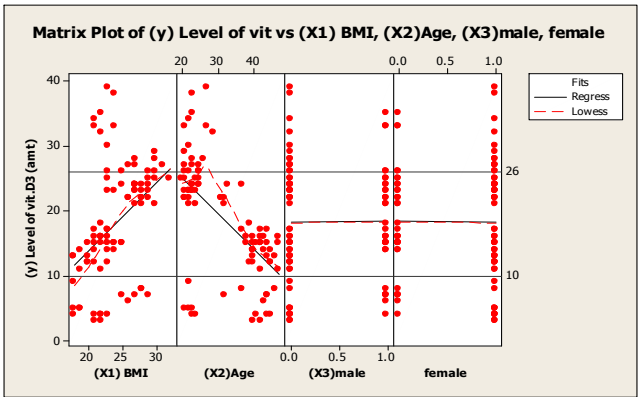

Figure 3: Correlation of vitamin D_3 with age, BMI, and gender

Figure (3) shows correlation of gender to the BMI, age and vitamin D3.

Effect of diet on serum vitamin D_3 and its interaction with age and BMI

There was correlation between diet (when divided meat, plant, and balanced), BMI, age and serum vitamin D_3.

Figure (4) shows that students who ingest meat and balanced diet had similar effect on serum vitamin D_3 and they had low level of vitamin, while student ingest more vegetables diet had high lower level of vitamin D_3.

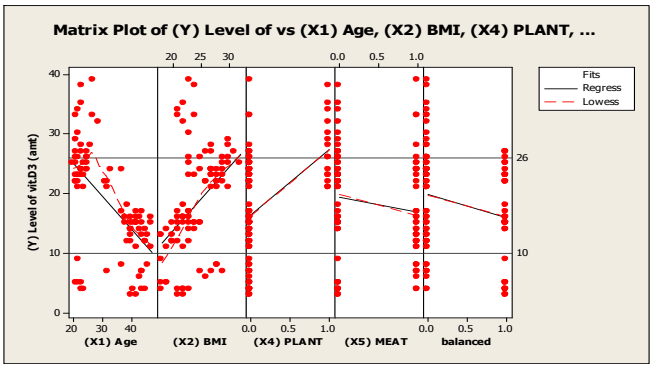

Figure 4: Correlation of vitamin D_3 with age, BMI, and diet habit.

Effect of level of education on serum vitamin D3 and its interaction with age and BMI

There was a correlation between gender, age BMI and serum vitamin D3.

Figure (5) shows that student's shifted from under graduate to post graduate there is a decrease in vitamin D_3 level.

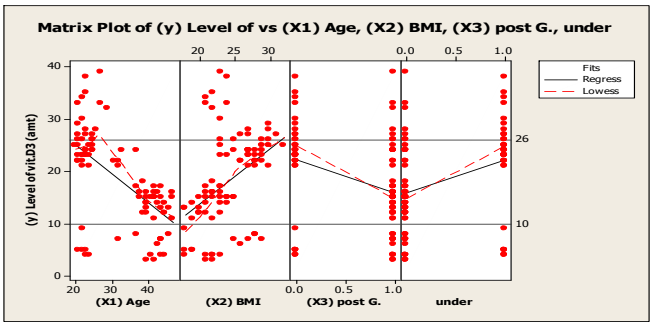

Figure 5: Correlation of vitamin D_3 with age, BMI, and level of education.

Table 3: The effect of walking exercise level, gender, diet, and education level on mean serum vitamin D_3

Variable	No of peoples	Mean Vitamin D3	P value
Walking exercise level[¥]			
≤ 30 min	11[A]	22.91 ± 1.37	0.0000
> 30 min	8[A]	29.75 ± 3.58	
No exercise	71[B]	16.34	
Gender			
Male	33	18.42 ± 7.95	0.941
Female	57	18.281 ± 9.323	
Diet			
Balanced	35	15.971 ± 7.943	0.0000
Meat	40	16.975 ± 8.499	
Plant [β]	15	27.467 ± 5.41	
Education Level			
Post graduate	56	15.93 ± 7.85	0.001
Under graduate	34	22.29 ± 8.96	
BMI [¥]			
Obese	7 [A]	25.857	0.007
Over weight	32 [A,B]	20.125	
Normal	51 [B]	16.176	

ANOVA analysis was done on sample studied and it showed that there is significant difference in the means of vitamin D_3 among level of education, Table 3.

Also the effect of BMI using level of education as predictor shows that undergraduate had higher mean serum vitamin D compared to postgraduate students.

[¥] Tukey test was done to evaluate in-group variation

[A,B] mean how show similar letter (A,B) do not show statistically different value of vitamin D when compared using post hoc tukey test.

BMI: body mass index

DISCUSSION:

Vitamin D_3 inadequacy show global pattern that emerge to surface in the recent decades with advance in detection and identification of serum vitamin D_3, its prevalent in all age groups especially the elderly owing to their reduce metabolism and dietary intake it has been estimated that up to one billion people around the world may have vitamin D deficiency or insufficiency [(7)].

In this study, the mean level of vitamin D_3 in the sample studied was 18.33 ± 8.8 ng/ml, and the prevalence of severe (true deficiency) and mild to moderate vitamin D were 18.9% and 61.1% respectively and 80% of individuals were vitamin D deficient, only 20% had optimal status of vitamin D_3.

This study had many limitations, the main project was not primarily aimed at studying vitamin D-related conditions; therefore, the amount of specific information pertaining to bone status and

vitamin D was limited. For instance, nutritional vitamin D intake was not quantified; neither measurements of osteoporosis or bone mass, nor other indicators of bone metabolism or structure, were obtained simultaneously, because it was a cross-sectional descriptive study, no temporal relationship between low vitamin D and the subsequent development of bone-related or other medical conditions could be obtained in this study.

Multiple studies have been carried out about the prevalence of vitamin D deficiency but they were mostly limited to a small sample size or assessed a specific age group (especially elderly), in a study was done in America to assess vitamin D status in a group of healthy free-living elderly people was determined by measuring dietary and supplemental vitamin D intakes and the plasma concentration, they found Plasma vitamin D was significantly lower in the elderly (15.5 ng/ml) compared to a younger control (29.1 ng/ml) population, they attribute this low values to inadequate consumption of supplementation of vitamin D and it was independent variable from seasonal variation , the prevalence of less than 8 ng/ml was 14.8% (39 patients) [8].

While in a study was done in Swiss population; mean vitamin D was 50 nmol/L (20 ng/ml) which is higher than the mean serum vitamin D, and in this study 6% were severe vitamin D deficient (<8 ng/ml) which is lower than our study, they attribute this low value because of age > 65 years, and outdoor < 30 minutes, around 95% of population with serum vitamin D less than 95 nmol/L (38 ng/ml) as well as genetic , environmental , number of studied individuals and longer duration of study. In these countries where vitamin D fortified foodstuffs (USA and some Scandinavian countries), prevalence of vitamin D deficiency is between 6 – 14.8% in different age groups. [9]

In other European countries where there was no vitamin D supplementation, deficiency is more prevalent, the studies which assessed middle-aged and elderly people showed vitamin D deficiency prevalence of 14% to 59.6% in these age groups [11,16,17] while vitamin D deficiency prevalence is much higher in Asian countries. [10]

The prevalence of severe vitamin D deficiency was 18.9% (below 25 nmol/l (10 ng/ml)) in this study, it was lower prevalence compared to other studied were done in Saudi Arabia in which vitamin D were measured in 104 Saudis, 44 Jordanians , and 17 Egyptians aged between 18 and 23 years and their results was 35%, 45%, and 53% below 10 ng/ml respectively, in another study done in Iran (Tehran) it found 40% of subjects with ≤ 10 ng/ml vitamin D_3.This difference might attributed to the following factors include sample size, duration of study, age, diet habit, and culture of society. [10]

Mean vitamin D_3 was 18.33 ng/ml with 95% CI (16.49 – 20.18) with standard deviation of 8.8 [95% CI 7.68 – 10.31], compared to different region in the Middle East like Saudi Arabia 11 - 12.9 ng/ml [12], in Jordon mean vitamin D_3 (male where 17.6 ng/ml and female 14.8 ng/ml) [8], while in Iran it was 8.9 ng/ml [8], compared to these studies our study show higher mean serum vitamin D_3. Turkey population show higher mean vitamin D_3 male with 38 ng/ml and female 24 ng/ml [8]. In our study male and female variation in serum vitamin D3 did not show statistically difference with male 18.4 ng/ml and female 18.2 ng/ml compared to the above studies which show significant variations, this may be caused by premenopausal female selected (young age) while the other studies show older females; while Swiss study had a similar to our results [9].

Most studies have shown higher prevalence of vitamin D_3 deficiency in the elderly with more severe deficiency [9,13,14,15] this also was seen in our study; this could be attributed to decreased amount ingested of vitamin D_3 with increase in age, environmental factors, genetic, sample size and duration of studied.

The mean age of healthy participant in this study was 32 years. Almost half of the patients were in the normal range of BMI, and third of them were in the overweight range , making only less 10% of them obese, and as the degree of body weight shifted from normal to obese so that mean vitamin D increased.

CONCLUSION:

Most studied subjects had mild to moderate vitamin D inadequacy. Factors like advance in age, lack of daily exercise, ingestion of meat diet, and low BMI carry a risk for severity of vitamin D deficiency. Gender did not show any effect on vitamin D variation.

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